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Mycoplasma pneumoniae—an emerging extra-pulmonary pathogen

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ABSTRACT

Mycoplasma is a well-recognised pathogen that colonises mucosal surfaces of humans and animals. *Mycoplasma pneumoniae* infects the upper and lower respiratory tracts of children and adults, leading to a wide range of respiratory and non-respiratory clinical conditions. *M. pneumoniae* infection is frequently considered in the differential diagnosis of patients with respiratory illnesses, and is commonly managed empirically with macrolides and fluoroquinolones. This contrasts with patients who present with non-respiratory symptoms in the context of a recent or current unrecognised *M. pneumoniae* infection, for whom this pathogen is rarely considered in the initial differential diagnosis. This review considers the microbiological, epidemiological, pathogenic and clinical features of this frequent pathogen that need to be considered in the differential diagnosis of respiratory and non-respiratory infections.

Keywords Diagnosis, epidemiology, extra-pulmonary infections, *Mycoplasma pneumoniae*, pathogenesis, review

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INTRODUCTION

Recognition of *Mycoplasma* as a bacterial pathogen took many years of intense clinical and research work. The first published report concerning *Mycoplasma* came from the French scientists Nocard and Roux [1], who in 1898 described the isolation, culture and infectivity of a microorganism associated with contagious bovine peri-pneumonia, later named *Mycoplasma mycoides*. No reports concerning *Mycoplasma* in humans appeared until 1942, when Eaton *et al.* [2] described the isolation of a filterable agent recovered from the sputum of patients with primary atypical pneumonia. This agent, later named the Eaton agent, was isolated from tissue cultures and infected rodents, with the latter subsequently developing a lung pathology similar to that observed in humans. Finland *et al.* [3] demonstrated that serum from patients with primary atypical pneumonia contained cold agglutinins capable of neutralising the Eaton agent. For many years, the Eaton agent was believed to be a virus particle rather than a bacterium [4]. The absence of a cell wall, which gives *Mycoplasma* a pleomorphic phenotype and the ability to pass through viral filters, as well as the difficulty of growing this organism in cell-free culture conditions, supported the hypothesis of the viral nature of the Eaton agent for 20 years. Important developments during the 1960s included the isolation of the Eaton agent from tissue culture cells and, more importantly, from cell-free cultures, thus demonstrating the bacterial nature of the Eaton agent [5]. Induction of atypical pneumonia in volunteers inoculated with purified isolates of the Eaton agent provided proof that this bacterial pathogen, later named *Mycoplasma pneumoniae*, was an aetiological agent of atypical pneumonia in humans [6].

M. pneumoniae is now known to be a frequent respiratory pathogen in children as well as in adults. *M. pneumoniae* infects the upper and lower respiratory tracts, leading to upper respiratory tract infection, bronchiolitis, tracheobronchitis, bronchitis and community-acquired pneumonia. It is also associated with asthma exacerbations [7]. Interestingly, *Mycoplasma* respiratory tract infections are associated with non-respiratory

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symptoms in many cases, manifesting in the skin, mucosas, central nervous system (CNS) and other tissues. Improved familiarity with clinical extrapulmonary manifestations of *M. pneumoniae* infection may improve diagnosis and help to ensure appropriate treatment.

MICROBIOLOGY

Mycoplasma belongs to the class Mollicutes, which includes organisms lacking the genes necessary to synthesise peptidoglycan cell walls. The class Mollicutes comprises four orders, five families, eight genera and 200 species [8,9]. Mollicutes are related phylogenetically to Gram-positive bacteria, based on 16S rRNA phylogenetic analysis. Despite the genetic relatedness of *Mycoplasma* to ancestral Gram-positive bacteria, the absence of a cell wall prevents successful staining with Gram's strain. The *Mycoplasma* chromosome is circular and *c.* 500 kb in size, making *Mycoplasma* one of the smallest autonomously replicating living organisms in nature [10].

The absence of a rigid cell wall makes *Mycoplasma* pleomorphic and able to cross filters that are otherwise permeable only to viruses. *Mycoplasma* may have a spherical shape under hypotonic conditions, and an irregular shape under hypertonic conditions; dehydration kills *Mycoplasma* [11,12]. All members of the *Mycoplasma* genus require sterols for growth. These essential components of the *Mycoplasma* membrane provide support to this osmotically fragile organism [13].

Mycoplasma genomic DNA lacks almost all the genes necessary for the biosynthesis of amino-acids, fatty acids, co-factors and vitamins, and the organism therefore depends on the host for a supply of metabolic precursors [11]. This limited biosynthetic potential means that mycoplasmas are obligatory parasites with strict host and tissue specificities [14]. Their host range includes plants, insects, animals and humans [15]. In humans, some colonising species behave as normal flora (e.g., *Mycoplasma salivarium*, *Mycoplasma orale*), while other species are clearly established pathogens (e.g., *M. pneumoniae*, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Mycoplasma fermentans* and, possibly, *Mycoplasma penetrans*) [9,16]. *M. pneumoniae* may elaborate capsular material around the cell membrane [17]. Little is known concerning capsular expression, the genes necessary for its biosynthesis, and its role in *M. pneumoniae* pathogenesis. Table 1 summarises the most important biological characteristics of *M. pneumoniae.*

PATHOGENESIS

The Mollicutes are primarily mucosa-associated organisms that reside in the host's respiratory and urogenital tracts in close association with epithelial cells, yet are located extracellularly [18]. There are several proposed mechanisms to explain pathogenicity, including competition for precursors, adherence to cells, fusion to cell membranes, cell invasion and cytotoxicity [11,14].

Cyto-adherence and its importance in pathogenesis

M. pneumoniae colonises the respiratory epithelium by attaching to cilia [19]. *Mycoplasma* cyto-adherence to the respiratory tract is the initial event leading to colonisation, infection and lung tissue damage [14]. P1, P30, P116 and HMW1–3 comprise a group of membrane proteins associated with *Mycoplasma* cyto-adherence [20–22], some of which concentrate on a single attachment tip organelle (Fig. 1) found at the surface of *Mycoplasma* [23–25]. Alteration or the absence of any of these proteins results in *Mycoplasma* becoming avirulent [26,27].

Cell invasion and pathogenesis

Although *M. pneumoniae* is primarily an extracellular pathogen that depends on close host–cell contact for survival, in-vitro studies have shown that it can penetrate cell membranes and invade cells [28]. Another species, *M. penetrans*, is also

Table 1. Bacteriological features of *Mycoplasma pneumoniae*

Taxonomy	Class Mollicute Family Mycoplasmataceae Order Mycoplasmatales Genus *Mycoplasma*
Phylogenetics	16S rRNA sequence analysis indicates that *Mycoplasma* split from *Streptococcus* *c.* 605 million years previously
Genome	816 kb in size Low G + C content Limited metabolic and biosynthetic genes
In-vitro growth	Requires complex media Sterols essential for membrane stability Low turbidity in culture
Features	Pleomorphic because of the absence of a cell wall Tip structure for attachment
Host	Humans

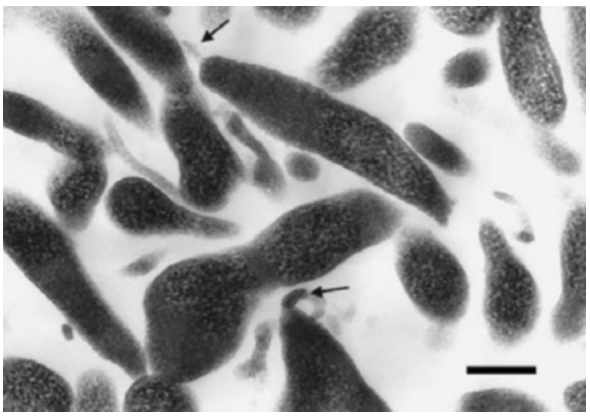

Fig. 1. Ultrastructural morphology of *Mycoplasma penetrans*. Transmission electron-microscopy image with negative staining showing elongated pleomorphic *Mycoplasma* cells grown in cell-free culture. Arrows indicate attachment tip organelle. Solid bar represents 200 nm (micrograph kindly provided by J. A. Giron, University of Arizona, USA).

able to invade tissue culture cells, and is a recognised pathogen that infects the genitourinary tract of patients with AIDS [29,30]. In-vitro evidence indicates that *Mycoplasma* can enter tissue culture cells within 2 h, and can remain intracellular for >7 days. Confocal microscopy has identified *Mycoplasma* in perinuclear regions and throughout the cell cytoplasm [31]. The interaction between *Mycoplasma* and epithelial cells triggers signals that induce recruitment of cytoskeletal proteins, including tubulin and α-actinin. Furthermore, it has been shown that *M. penetrans* also binds the extracellular matrix protein fibronectin, suggesting that signalling mediated by integrin–fibronectin may induce internalisation of *Mycoplasma* [32].

Cytotoxicity mediated by *Mycoplasma*

Mycoplasma internalisation into host cells is not necessary for the initiation of local cytotoxic events and clinical manifestations of disease. Cytopathic changes can be related to the local damage following cyto-adherence. Close contact between *Mycoplasma* and host tissue allows local disruption and cytotoxicity through the release of enzymic and cytolytic metabolites directly on to the cell [14]. Fusion of *Mycoplasma* membrane to host-cell membrane may result in the release of hydrolytic enzymes produced by *Mycoplasma*, as well as the insertion of bacterial membrane components into the host cell membrane. *Mycoplasma* nucleases have been shown to induce inter-nucleosomal DNA fragmentation in cultured cells [33], and an ADP-ribosyltransferase toxin with homology to pertussis toxin was reported to induce vacuolisation of epithelial cells [34]. Several epithelial cell proteins are targeted by the vacuolising toxin, although none have so far been identified.

Immune response and cytokine production

Mycoplasma activates the immune system by inducing B- and T-lymphocyte proliferation, secretion of major histocompatibility complex class I and II proteins, and release of multiple cytokines (e.g., interleukins, interferons, tumour necrosis factor and colony-stimulating factors). These effects may result in local or systemic manifestations in the infected host. Cytokines are important mediators of inflammation in respiratory and non-respiratory tissues, as demonstrated by in-vivo and in-vitro studies (Table 2). *Mycoplasma* spp. induce cytokines in tissue culture cells, experimental animals and humans [19,35–37]. *Mycoplasma*-mediated cytokine release in infected children is associated with worsening asthma symptoms [7]. Studies in mice indicate that the T-helper 1 type of immune response

Table 2. *Mycoplasma*-mediated cytokine secretion and organ-system effects

Cytokine	Effect	Reference
IL-1β	Elevation associated with AHR	[38]
	Elevated in BALF and serum in MP	[15]
IL-2	Elevated in BALF and serum in MP	[15]
	Suggested role in development of pulmonary lesions	[43]
IL-4	Elevated production by Mast cells *in vitro*	[79]
	Involved in AHR in asthmatics	[80]
	Elevated in BALF and serum in MP	[15]
IL-5	Elevation associated with AHR in children	[7]
IL-6	Elevated production by Mast cells *in vitro*	[79]
	Elevation associated with AHR	[38]
	Elevation associated with CNS complications	[63]
	Elevated in BALF and serum in MP	[38]
IL-8	Increase neutrophil influx to the alveolar spaces in MP	[81]
	Elevation associated with CNS complications	[63]
	Elevated in AHR patients	[82]
IL-12	Elevation associated with AHR	[38]
IL-18	Enhances NK cell cytotoxicity and T-lymphocyte activation	[37]
	Elevation associated with CNS complications	[63]
	Serum levels increased in acute MP	[37]
IFN-δ	Elevation associated with AHR	[38]
	Increased macrophage and NK cell activity	[16,42]
TNF-α	Induced secretion by lung cells *in vitro*	[15]
	Elevation associated with AHR	[38]
	Elevated production by mast cells *in vitro*	[79]
TGF-β_1	Induced in airway epithelial cells	[39]
RANTES	Induced in airway epithelial cells	[39]

RANTES, normal T-cell-expressed and secreted chemokine; MP, *Mycoplasma* pneumonia; CNS, central nervous system; AHR, airway hyper-responsiveness; BALF, bronchoalveolar lavage fluid; IL, interleukin; IFN, interferon; TNF, tumour necrosis factor; TGF, transforming growth factor; NK, natural killer.

induced by *Mycoplasma* is responsible for increased airway obstruction and elevated airway hyper-responsiveness [38]. The ciliated airway epithelium is the primary site of *Mycoplasma* infection, and is the principal source of a variety of cytokines with a pro-inflammatory role [39]. Evidence suggests that cell surface lipoproteins are the preferential target of the humoral immune response [40].

Evasion and suppression of the immune response

The major mechanisms implicated in the evasion of the immune response by *Mycoplasma* include molecular mimicry, survival within cells and phenotypic plasticity. Some *Mycoplasma* spp. may undergo changes in the lipoprotein repertoire expressed in the cell membrane as a way of coping with a fluctuating environment and the host immune response [40]. Furthermore, *M. pneumoniae* may induce transient depression of T-lymphocyte function and depletion of $CD4^+$ T-cells [41,42]. Induction of transient anergy has also been described during the acute phase of *Mycoplasma* infection [43]. Similarly, a study in children acutely infected by *M. pneumoniae* reported a temporary suppression of the immune system by mechanisms as yet unknown [44].

EPIDEMIOLOGY

M. pneumoniae can be transmitted by aerosols from person to person. Individuals with active *Mycoplasma* infection carry organisms in the nose, throat, trachea and sputum, with transmission facilitated by coughing. The incubation period varies from 1 to 3 weeks, although it is sometimes as short as 4 days [9,45]. During epidemics, the presence of different subtypes may explain the lack of a protective immune response against subsequent infection [46]. Studies in outpatient clinics in Seattle, WA, USA reported that *M. pneumoniae* infection rates varied from 2% in endemic years to 35% in epidemic periods. Epidemics occurred every 4–7 years. A higher proportion of children aged 5–9 years developed pneumonia than did adolescents aged 15–19 years [47]. *Mycoplasma* outbreaks tend to occur in crowded environments or institutions such as hospitals, military camps and college dormitories [48,49]. Studies using PCR screening have indicated that a possible carrier status may provide a reservoir for these organisms. Although *M. pneumoniae* is not part of the normal flora, and its presence is frequently associated with infection, the microorganism may persist inside the host's respiratory tract for variable periods even after the clinical manifestations have resolved [50]. Furthermore, *M. pneumoniae* infection tends to be more severe among immunosuppressed individuals, including individuals with humoral immune defects.

CLINICAL MANIFESTATIONS

M. pneumoniae infection most commonly affects the upper and lower respiratory tracts. Upper respiratory tract symptoms include sore throat, hoarseness, fever, cough, headache, chills, coryza, myalgias, earache and general malaise. Infections of the lower respiratory tract generally manifest with a cough, sometimes with dyspnoea, adenopathy, wheezing and, rarely, with respiratory failure. Fulminant infections are uncommon. Although *M. pneumoniae* infections are usually mild, and many are asymptomatic, they are not always self-limiting [9]. *M. pneumoniae* causes up to 40% of cases of community-acquired pneumonia, but *M. pneumoniae* respiratory tract infections are also associated with a wide range of extra-pulmonary manifestations, including neurological, cardiac, dermatological, musculoskeletal, haematological and gastrointestinal symptoms [8,41,51,52] (Table 3).

The development of new techniques for the detection of *M. pneumoniae* has revealed that this organism can also be found in extra-pulmonary tissues [16], with *c.* 25% of individuals infected with *M. pneumoniae* experiencing extra-pulmonary complications [8]. Extra-pulmonary manifestations could occur before, after, during or in the absence of respiratory symptoms. Extra-pulmonary manifestations may occur not less than 3 days after the onset of respiratory disease, and for 2–3 weeks after the respiratory disease has resolved [53]. Whether infection itself or post-infection inflammation is responsible for the extra-pulmonary clinical manifestations is currently under investigation.

While *M. pneumoniae* most commonly infects the respiratory tract, infections caused by *Mycoplasma* spp. other than *M. pneumoniae* have been

Table 3. Clinical manifestations caused by or associated with *Mycoplasma pneumoniae* infection

Respiratory tract conditions directly related to *M. pneumoniae* infection
Tonsillitis
Rhinitis
Tracheobronchitis
Pharyngitis
Bronchiolitis
Croup
Bronchopneumonia
Atypical pneumonia
Asthma
Clinical conditions associated with *M. pneumoniae* infection by system
Neurological
Encephalitis
Meningoencephalitis
Cerebral ataxia
Aseptic meningitis
Transverse myelitis
Guillain–Barré syndrome
Polyradiculitis
Peripheral neuropathy
Optic neuritis
Cranial nerve palsies
Stroke
SIADH
Renal
Glomerulonephritis
Renal failure
Tubulointerstitial nephritis
IgA neuropathy
Dermatological
Erythematous maculo-papular and vesicular rashes
Generalised ulcerative stomatitis
Bullous exanthems
Stevens–Johnson syndrome
Erythematous maculo-papular rash
Vesicular rash
Erythema nodosum
Pityriasis rosea
Toxic epidermal necrolysis
Bullous erythema multiforme
Subcorneal pustular dermatosis
Ophthalmological
Conjunctivitis
Anterior uveitis
Retinitis
Retinal haemorrhages
Iritis
Optic disk swelling
Musculoskeletal
Arthralgias
Septic arthritis
Myalgias
Acute rhabdomyolysis
Haematological and cardiovascular
Haemolytic anaemia
Intravascular coagulation
Aplastic anaemia
Thrombotic thrombocytopenic purpura
Urticarial vasculitis
Leukocytolastic vasculitis
Henoch-Schoenlein purpura
Pericarditis
Myocarditis
Pericardial effusion
Raynaud phenomenon
Gastrointestinal
Diarrhoea
Cholestatic hepatitis
Pancreatitis
Hypoechoic lesions in spleen

Adapted from [8,41,52,57,60,82,84–87].
SIADH, syndrome of inappropriate anti-diuretic hormone secretion

described in blood, brain tissue, cerebrospinal fluid (CSF), skin, the urogenital tract, heart and joints [9,16,29,54,55].

Skin and mucosal infections

Among patients with *M. pneumoniae* infection, *c.* 25% may have dermatological manifestations, making these some of the most common complications of this infection [43]. A wide range of skin and mucosal manifestations has been described in the literature (Table 3). There is a well-known association between *Mycoplasma* and Stevens–Johnson syndrome, erythema multiforme and toxic epidermal necrolysis [56,57]. *M. pneumoniae* is the most common infectious agent associated with Stevens–Johnson syndrome [58,59]. Some cases of Stevens–Johnson syndrome were reported to exclusively affect mucosal membranes, leaving the skin intact [57,59]. It is unclear at present whether this entity is a variant of Stevens–Johnson syndrome or a new entity. Patients with oral, as well as genitourinary, mucosal lesions, generally manifest with fever and generalised fatigue. Antimicrobial therapy rapidly resolves the clinical condition.

The exact mechanism of skin and mucosal disease is unknown, but immune complex-mediated vascular injury, cell-mediated immune response and cytotoxic injury to epithelial cells, and autoimmune mechanisms have all been suggested [57]. While *M. pneumoniae* has been detected directly in cutaneous lesions, there is no evidence that *Mycoplasma* causes such lesions directly.

CNS manifestations

CNS manifestations are the most common extrapulmonary complications of *M. pneumoniae* infection. Encephalitis and meningoencephalitis are most common, followed by polyradiculitis and aseptic meningitis [41,51]. Frequently, a manifest respiratory infection precedes the CNS symptoms. The mean interval between the onset of respiratory symptoms and CNS manifestations is 9.6 days (range 2–14 days) [41,52]. *M. pneumoniae* infection should be routinely considered in the differential diagnosis of patients with CNS manifestations, especially if associated with pneumonia [60]. Table 3 lists the neurological conditions associated with *Mycoplasma* infection.

Among serologically confirmed *M. pneumoniae* infections that require hospitalisation, *c.* 1–10% are associated with neurological manifestations. The overall incidence is <0.1%, although the exact incidence of *M. pneumoniae*-associated

neurological complications remains unknown because of the absence of an appropriate diagnostic test.

M. pneumoniae is a major cause of encephalitis in children. Children aged <10 years are affected more frequently than adults. Severe presentations require intensive care management in up to 30% of cases with CNS involvement. Long-term neurological sequelae are documented in about one-third of serologically confirmed cases of encephalitis [61]. A prospective 5-year study of children with acute encephalitis found evidence of *M. pneumoniae* infection in 31% of all cases, with *M. pneumoniae* being the probable cause of encephalitis in 6.9% of cases, based on PCR detection of *Mycoplasma* DNA in CSF and positive serological results. Respiratory symptoms were absent in 36% of patients with probable *M. pneumoniae* encephalitis [62].

Acute transverse myelitis and acute disseminated encephalomyelitis are among the most severe CNS manifestations of *M. pneumoniae* infections. CNS symptoms and signs associated with *M. pneumoniae* infections usually resolve completely; however, a persistent neurological deficit has been described in up to one-third of patients [41,60].

The pathogenesis of CNS disease associated with *M. pneumoniae* remains unknown, and further research is underway to elucidate the possible mechanism(s) of pathogenesis. Direct invasion of the CNS by *M. pneumoniae* has been implicated in early-onset encephalitis, but the only evidence for this has been PCR-based detection of *M. pneumoniae* in CSF [52,53,63]. While detection of *M. pneumoniae* in the CNS may be the result of migration of antigen-presenting cells from pulmonary sites carrying *Mycoplasma* DNA [64], it is known that *M. hominis* actively crosses the blood–brain barrier to cause brain abscesses [65]. Neurotoxin may also be implicated in the pathogenesis of CNS disease, and an *M. pneumoniae* virulence factor with ADP-ribosyltransferase activity and homology to pertussis toxin has been described that leads to epithelial cell damage *in vitro* [34]. This toxin may have systemic effects that include the CNS. More studies are necessary to elucidate the role of this toxin in *Mycoplasma* extra-pulmonary disease. Immune response-mediated damage, by bacteria-induced immunosuppression, immune complexes or autoimmune mechanisms, may better explain late-onset encephalitis. Molecular mimicry and the production of anti-human antibodies have been associated with *Mycoplasma* infections and antibodies against brain tissue antigens that may contribute to the neurological injury [66]. Lastly, thrombosis and a hyper-coagulable state may lead to intravascular coagulation and thromboembolic CNS complications in association with *Mycoplasma* [41,52].

The peripheral nervous system may also be involved during *M. pneumoniae* infection in children [67] and young adults. Studies in individuals aged <35 years with Guillain–Barré syndrome have revealed a significant association with *M. pneumoniae* infection [68].

DIAGNOSIS

M. pneumoniae infections cannot be diagnosed by clinical findings alone, especially when they present with extra-pulmonary symptoms. Before the availability of new technologies, cold agglutinins were used to confirm a diagnosis of *M. pneumoniae* infection. Cold agglutinins are IgM antibodies directed to antigen 1 on erythrocytes. They are produced 1 or 2 weeks after infection in 50% of patients and may persist for several weeks. Lack of sensitivity and specificity render cold agglutinins irrelevant for diagnosis, as they may also be present in infections caused by viruses and other bacteria [52,69,70]. While culture is considered to be the reference standard for diagnosis, it is expensive and time-consuming, and requires specialised media and technical expertise. Furthermore, culture is not available except in reference laboratories or large medical centres (Table 4). Diagnosis of *M. pneumoniae* infection is usually performed by serological methods, such as passive agglutination, complement fixation and ELISA. A combination of PCR and serology is recommended for reliable diagnosis [71]. Serological tests for anti-*Mycoplasma* antibody represent the most common method for retrospective diagnosis of *Mycoplasma* infections. Evidence of seroconversion by collection of acute and convalescent sera is the optimal method for retrospective *Mycoplasma* diagnosis. Seroconversion is defined as a four-fold increase in titre between acute and convalescent sera, or a single high anti-*Mycoplasma* complement fixation antibody titre of >1:128. False-positive results caused by cross-reactions between antigens can occur.

Table 4. Diagnostic methods for *Mycoplasma pneumoniae*

Method	Features
Culture	Reference standard method
	Not used routinely in clinical practice
	Sensitivity no more than 60%
	Specificity 100%
	Disadvantages: laborious, expensive, long incubation periods
PCR	Amplification of specific *Mycoplasma* DNA fragments
	Advantages: may process histological samples, fluid, serum
	Report available faster than serology
	Does not require viable bacteria
	Disadvantages: unable to distinguish colonisation from infection
Serology	Advantages: easy to collect and to transport samples
	Sensitivity 90%
	Specificity 88% with titres >32 indicative of recent infection
	Disadvantages: time-consuming, cross-reactions with other *Mycoplasma* spp.
Other	Antigen detection by immunofluorescence, agglutination, immunoblotting
	Low sensitivity and high cross-reactivity

Adapted from [41,70–73,87].

Serological testing is often hampered by inter-species cross-reactions and even non-specific reactions [41,72]. The sensitivity and specificity of passive agglutination with single serum samples varies with the titre cut-off value used. It is suggested that a titre of 1:80 or 1:160 is useful for the diagnosis of *M. pneumoniae* infection in children. Passive agglutination serology using paired sera shows good agreement with PCR results [71]. ELISA is more sensitive than culture for detecting acute infection, has sensitivity comparable to PCR [71], but may be less sensitive than passive agglutination [71]. Complement fixation tests, indirect immunofluorescent assays and particle agglutination assays have low sensitivity and specificity [52].

PCR has been recommended for more sensitive detection of *M. pneumoniae*, especially for patients with neurological and other extra-pulmonary manifestations [41]. PCR uses the same specimens as does culture, but may also detect *Mycoplasma* in tissue processed for histological examination. The advantages of PCR include high sensitivity and specificity, rapid results, and no requirement for viable microorganisms [8]. However, PCR assays may overestimate the incidence of *Mycoplasma* infections and, at present, there is no standardised diagnostic method [72,73].

TREATMENT

While there is no disagreement concerning the optimum antibiotic management of *M. pneumoniae* respiratory tract infections, controversy and limited clinical evidence characterises the current situation concerning management of non-pulmonary conditions associated with *M. pneumoniae*.

Antibiotic management of *Mycoplasma* respiratory infections

The absence of a cell wall renders these organisms insensitive to β-lactam antibiotics. However, antibiotics acting at the level of protein synthesis or DNA modification molecules are highly effective. Macrolides, tetracyclines and fluoroquinolones eliminate *Mycoplasma* efficiently both *in vivo* and *in vitro*. Macrolides are the antibiotics of choice for treating *M. pneumoniae* infections in both adults and children. New macrolides are better-tolerated, require fewer doses and have a shorter treatment duration than older compounds.

In the ambulatory setting, it is more practical to provide therapy empirically. However, if the infection requires hospitalisation and the patient has risk-factors, e.g., an underlying condition, or an unfavourable prognosis, diagnostic testing is recommended [74]. Use of tetracycline and fluoroquinolones is limited to adult patients or to patients with an allergy to macrolides. Tetracyclines should not be used in children aged <8 years. Azithromycin is given at recommended doses for 5 days. Other macrolides such as clarithromycin and erythromycin, as well as tetracyclines and fluoroquinolones, usually require longer courses. A potential problem in the antimicrobial management of *M. pneumoniae* infections is the emergence of macrolide resistance, reported initially in Japan during 2000 [75]. Treatment of children with fluoroquinolones may be possible; however, these agents are not yet approved for use in children by the Federal Drug Administration [76]. While *M. pneumoniae* infections in the upper respiratory tract may improve following antibiotic treatment, this is not generally recommended, as such infections are usually self-limiting. Some clinicians recommend treatment of acute tonsillo-pharyingitis to prevent the risk of recurrence of respiratory illness [77].

Management of non-respiratory conditions associated with *Mycoplasma* infections

Controversies in the management of non-respiratory conditions associated with *M. pneumoniae* infections result from the limited knowledge of

their pathogenesis. While some extra-pulmonary conditions may be caused by a post-inflammatory response to *M. pneumoniae* infection, other conditions may result from direct tissue damage caused by this organism. Steroids have been used in selected patients with severe CNS syndromes, based on the presumed role of cytokines in inflammation, despite the absence of any objective prospective evaluation in clinical trials [41,52,63]. Case reports suggest that high-dose steroid therapy may reverse neurological manifestations in children. Aggressive therapy with steroids and high-dosage immunoglobulins in children was reported to improve outcome in cases of stroke related to *M. pneumoniae* infection [53]. Even severe cases of *M. pneumoniae* pneumonia in children also benefit from the use of steroids in conjunction with antibiotics [43].

In addition to steroids, other therapies, including plasmapheresis, plasma exchange and intravenous IgG, have been used to treat patients with severe CNS complications. None of these strategies has been tested in randomised double-blind clinical trials, and their benefit therefore remains unclear. Plasmapheresis was reported to be effective in cases of transverse myelitis or polyradiculitis [60]. Despite the absence of evidence, it seems reasonable to consider the use of immunomodulatory therapies, together with antibiotics, in severe cases.

The use of antibiotics for treating CNS conditions associated with *M. pneumoniae* infection is also reported to have variable results. Treatment with macrolides, tetracyclines, fluoroquinolones and chloramphenicol is limited to case reports involoving severe CNS conditions associated with *Mycoplasma* infections [78], and an actual benefit from antibiotic therapy was not demonstrated in most of the cases. The rationale for antibiotic use is based on the PCR-based detection of *Mycoplasma* DNA in CSF, and the assumption that this DNA is evidence of viable *M. pneumoniae* cells growing in the CNS. However, microbiological studies have failed to obtain positive *M. pneumoniae* cultures from CSF, which suggests that other mechanisms of pathogenesis may be involved.

Until more information is available concerning the pathogenesis of *M. pneumoniae*-associated extra-pulmonary conditions, it seems that supportive treatment remains the most important management approach [45], with the use of steroids and antibiotics being considered on an individual basis.

CONCLUSIONS

Mycoplasmas are fastidious microorganisms that were first identified a century ago and have subsequently been studied extensively both *in vivo* and *in vitro*. Advances in laboratory technology have significantly improved the diagnosis of *M. pneumoniae* respiratory infections and have increased the number of previously unrecognised extra-pulmonary conditions associated with *M. pneumoniae* infection. However, many questions remain unanswered concerning pathogenesis, the role of host factors in clinical presentation, and the medical management of extra-pulmonary conditions. While clinical and experimental evidence has accumulated concerning the role of *M. pneumoniae* in activating pro-inflammatory molecules and tissue inflammation, little is known with respect to the effect of newly described *M. pneumoniae* virulence factors on direct tissue damage or systemic cytotoxicity. Management of extra-pulmonary conditions is controversial, as neither antibiotics nor anti-inflammatory drugs have a clearly demonstrated clinical benefit. Basic research on the molecular biology of *Mycoplasma* infection will be important in gaining an understanding of the pathogenesis of the diverse clinical conditions associated with these organisms. Furthermore, clinical studies on the epidemiology and clinical management of extra-pulmonary conditions will be essential to better identify patients with conditions that may benefit from antibiotic therapy and those that may benefit from immunomodulatory therapies.

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